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Brief Research Communication

Identification of a BglI Polymorphism of Catechol-O-Methyltransferase (COMT) Gene, and Association Study With Schizophrenia

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Several linkage studies suggested chromosome 22q11–13 may harbor susceptible genes for schizophrenia. Catechol-O-methyl-transferase (COMT), which is involved in the metabolism of catecholamines, was mapped to 22q11 and is considered a possible candidate gene for schizophrenia. Recently, we identified a polymorphic marker, a single nucleotide C insertion at the 3′ untranslated region of the COMT gene, which obliterates a *BglI* site. Using this *BglI* polymorphism, we conducted a case-control association study in Chinese patients with schizophrenia. No significant differences of allele and genotype frequencies were noted between patients (N = 177) and controls (N = 99). When patients were subgrouped according to sex, no significant differences of genotype and allele frequencies were noted in either male or female patients compared to normal controls. Our results do not support an association between the *BglI* polymorphism of COMT gene and schizophrenia.

KEY WORDS: schizophrenia, association, COMT, *BglI* polymorphism

INTRODUCTION

Schizophrenia is a severe chronic disease, with a prevalence of about 1% in the general population. Family, adoption, and twin studies support genetic components of schizophrenia. However, the mode of inheritance is uncertain [Tsuang et al., 1991]. Currently, several approaches are being used to map the susceptible genes for schizophrenia, including linkage analysis, association study, and direct sequencing of candidate genes.

Several linkage studies suggested potential linkage of chromosome 22q11–13 with schizophrenia [Coon et al., 1994; Pulver et al., 1994a; Lasseter et al., 1995; Schwab et al., 1995; Vallada et al., 1995]. However, several replication studies did not find similar results [Polymeropoulos et al., 1994; Pulver et al., 1994b; Kalsi et al., 1995]. Nevertheless, studies of velo-cardio-facial syndrome (VCFS) supported the involvement of the long arm of chromosome 22 with schizophrenia. VCFS is a congenital malformation syndrome characterized by cleft palate, heart disease, and typical faces. Cytogenetic studies revealed that VCFS is associated with a microdeletion of chromosome 22q11 [Kelly et al., 1993]. Patients with VCFS usually have learning difficulties in childhood, and a higher rate of psychosis after adolescence [Scambler et al., 1992; Shprintzen et al., 1992; Pulver et al., 1994c], suggesting that some genes relevant to the behavioral problems in VCFS patients may reside in this region. Moreover, Lindsay et al. [1995] and Karayiorgou et al. [1995] reported that interstitial deletions of chromosome 22q11 were detected in a population with schizophrenia. These studies suggested that chromosome 22q11–13 may harbor some defective genes that may increase liability to schizophrenia.

Catechol-O-methyltransferase (COMT, EC 2.1.1.6) is involved in the metabolism of the catecholamine neurotransmitters, and was mapped to chromosome 22q11. COMT has been proposed to be involved in the pathogenesis of mental disorders in light of the biogenic amine hypothesis of mental disorders [Carlsson, 1988]. Dunham et al. [1992] proposed that psychosis in VCFS

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patients may be due to haploinsufficiency of the COMT gene in deleted 22q11. In this communication, we report identification of a *BglI* polymorphism of the COMT gene by direct sequencing of the genomic DNA, and development of a PCR-based restriction analysis to facilitate genotyping. We also conducted a population-based case-control association study of schizophrenia in a Chinese population using this polymorphic marker.

PATIENTS AND METHODS

One hundred and seventy-seven Chinese patients fulfilling the DSM-III-R diagnostic criteria of schizophrenia were recruited from two private psychiatric hospitals in the Taipei area, Taiwan. The patient group consisted of 95 males and 82 females, with a mean age of 47 years. The controls were adult nonpsychiatric patients recruited from a community general hospital in the same area as the psychiatric hospitals. The control group consisted of 55 males and 45 females, with a mean age of 45 years.

Biotinylated sense primer (5′-TGGGCACCTCTGACCTCTCAC-3′) and antisense primer (5′-CTGGGCACCTCTGACCTCTCA-3′) were used to PCR-amplify exon 6 and flanking sequences of the COMT gene for direct solid-phase sequencing [Hultman et al., 1989]. PCR conditions were as follows: after an initial incubation at 95°C for 5 min, amplification (30 cycles) was performed with denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min.

An insertion of a cytosine nucleotide at the 3′ untranslated region (UTR) of the COMT gene was identified, as illustrated in Figure 1. The C insertion is amid a stretch of cytosine sequences, 12 bp downstream from the TGA stop codon of exon 6 of the COMT gene [Tenhunen et al., 1994]. The C insertion obliterates a *BglI* restriction site; hence, a PCR-based restriction analysis was developed to facilitate genotyping.

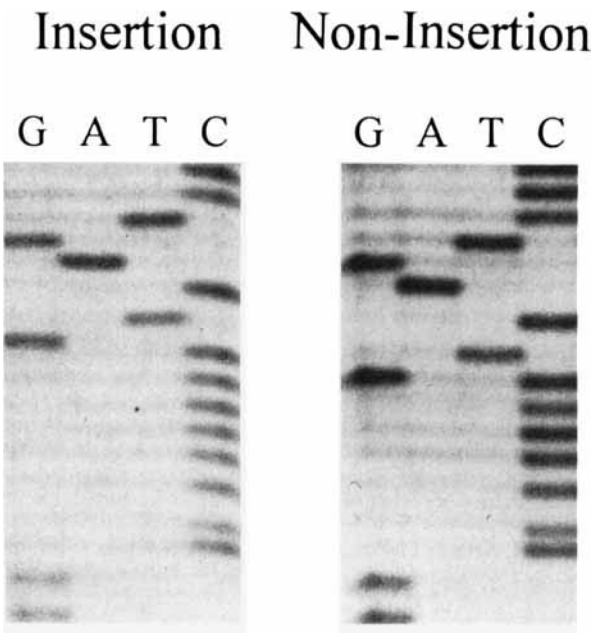

Fig. 1. Identification of a C insertion at the 3′ UTR of the COMT gene by direct solid-phase sequencing. Insertion indicates a homozygous subject with a cytosine nucleotide insertion. Noninsertion indicates a homozygous individual lacking C insertion in both alleles.

PCR amplification of exon 6 from genomic DNA was performed essentially as described, except that the biotinylated sense primer was replaced with nonbiotinylated primer (5′-CTGGGCACCTCTGACCTCTCA-3′). After amplification, an aliquot (10 μl) of PCR product was incubated with 5 U of *BglI* (New England Biolabs Inc., Beverly, MA) in a volume of 15 μl at 37°C overnight. The digested PCR fragments were subjected to electrophoresis in 3% agarose gel, stained with ethidium bromide, and visualized under UV light. The results are illustrated in Figure 2.

RESULTS

The allele and genotype frequencies of the *BglI* polymorphism of patients and normal controls are shown in Table I. The C insertion allele is common in the Chinese population, with a frequency of 0.45. The genotype distributions in patients ($\chi^2 = 3.2$, $df = 1$, $P = 0.07$) and controls ($\chi^2 = 0.98$, $df = 1$, $P = 0.32$) did not deviate from the Hardy-Weinberg equilibrium. There was no significant difference of genotype frequencies ($\chi^2 = 3.48$, $df = 2$, $P = 0.18$) and allele frequencies ($\chi^2 = 2.68$, $df = 1$, $P = 0.10$) between schizophrenic patients and controls. When patients were divided according to sex, there was still no significant difference of genotype frequencies ($\chi^2 = 3.07$, $df = 2$, $P = 0.22$) or allele frequencies ($\chi^2 = 2.13$, $df = 1$, $P = 0.14$) between male schizophrenic patients and male controls. Also, no differences of genotype frequencies ($\chi^2 = 0.87$, $df = 2$, $P = 0.65$) or allele frequencies ($\chi^2 = 0.59$, $df = 1$, $P = 0.44$) were detected between female patients and female controls.

DISCUSSION

In the present study, we report no association between a *BglI* polymorphism at the COMT gene and schizophrenia in a Chinese population from Taiwan. Our results do not support potential linkage of the COMT locus and schizophrenia, as suggested by several linkage studies. Our study is consistent with a recent report by Nimgaonkar et al. [1995], who found no association between a CA dinucleotide polymorphic marker of the interleukine 2 receptor β chain (IL-2Rβ)

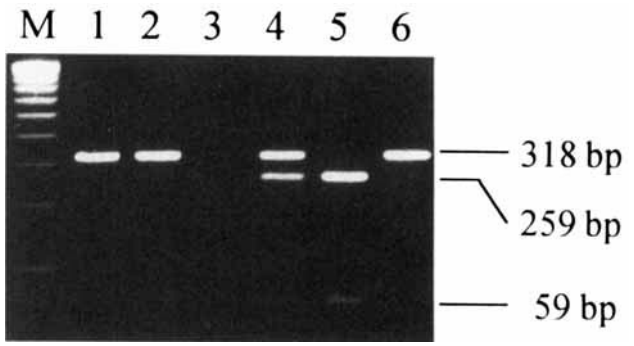

Fig. 2. PCR-based genotyping of *BglI* polymorphism of C insertion. **Lanes 1, 2,** and **6** are homozygotes of the C insertion, who are *BglI* noncutters; **lane 5** is a homozygote of noninsertion, showing PCR fragments of 259 bp and 59 bp after digestion with *BglI*; **lane 4** is a heterozygote of the C insertion, showing 318-bp, 259-bp, and 59-bp PCR fragments after digestion with *BglI*.

TABLE I. Genotype and Allele Frequencies of *BglI* Polymorphism in Schizophrenic Patients and Controls*

		Genotype			Allele frequency	
	N	+/+	−/+	−/−	+	−
Schizophrenia	177	33 (0.19)	100 (0.56)	44 (0.25)	0.47	0.53
Male	95	17 (0.18)	56 (0.59)	22 (0.23)	0.47	0.53
Female	82	16 (0.19)	44 (0.54)	22 (0.27)	0.46	0.54
Controls	99	27 (0.27)	54 (0.55)	18 (0.18)	0.55	0.45
Male	48	14 (0.29)	27 (0.56)	7 (0.15)	0.57	0.43
Female	51	13 (0.25)	27 (0.53)	11 (0.22)	0.52	0.48

*N, number of individuals; +, *BglI* cutting allele; −, *BglI* noncutting allele.

gene and schizophrenia. The IL-2Rβ gene was mapped to 22q12–13, and was suggested as a possible candidate gene for schizophrenia by linkage studies [Pulver et al., 1994a; Coon et al., 1994] and biochemical studies [Ganguli and Rabin, 1989; Rapaport et al., 1989, 1993]. Recently, Moises et al. [1995] reported a potential linkage disequilibrium between the D22S278 locus on the long arm of chromosome 22 and schizophrenia. D22S278 is located at least 45 cM distal to the COMT locus. Thus, it is still likely that some unidentified genes in the region that are tightly linked to D22S278 may underlie susceptibility to schizophrenia in some subsets of patients.

Although we did not find association between the *BglI* polymorphism of the COMT gene and schizophrenia in the present study, the high heterozygosity (55%; estimated polymorphic information content is 0.37) and convenient genotyping method of the *BglI* polymorphism of the COMT gene should be useful for mapping other genes in this region.

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